Supporting Information:

Substrate Binding Regulates Redox Signaling in Human DNA Primase

Elizabeth O'Brien,[1,2] Marilyn E. Holt,[3] Lauren E. Salay,[3] Walter J. Chazin,[3*] and Jacqueline K. Barton[1*]

Contents:

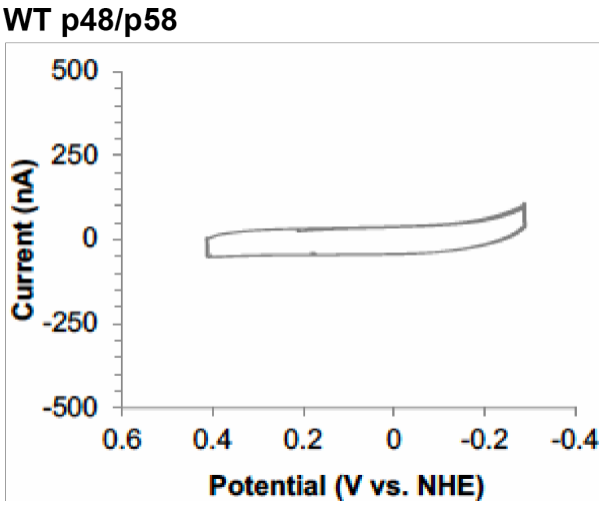

Figure S1. Electrochemically unaltered human p48/p58 displays no significant redox activity in CV scans, suggesting that the resting state of the enzyme is either not bound to DNA or bound in a manner that does not promote coupling of the [4Fe4S] cluster to the DNA bases for redox signaling. A small peak associated putatively with the $[3Fe4S]^{+}$ degradation product is observable using the more sensitive electrochemical technique, Square Wave Voltammetry (SWV), but no significant redox activity from the $[4Fe4S]^{2+/3+}$ couple is observed.

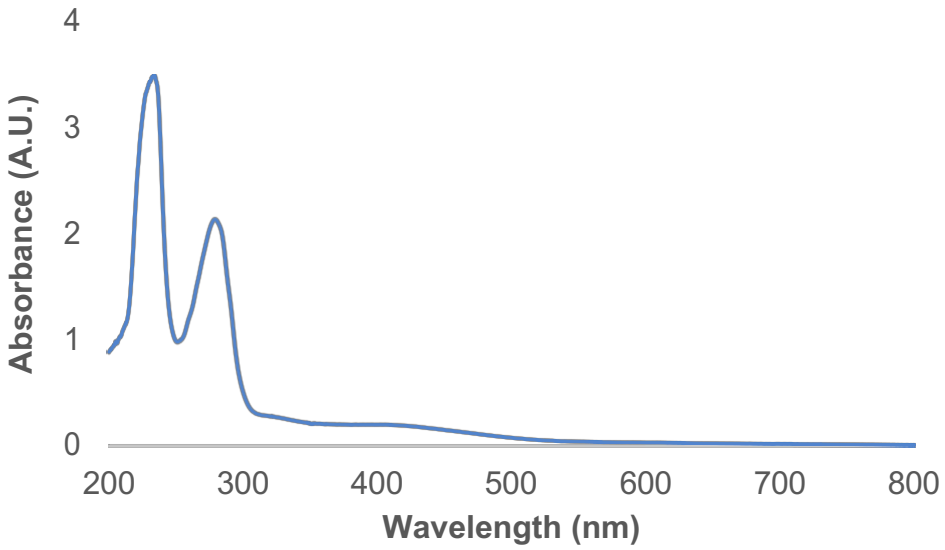

Figure S2. UV-Visible spectrum of p48/p58 after exchange into 20mM HEPES, pH 7.2, 150mM NaCl, 5% glycerol from the original Tris storage buffer.

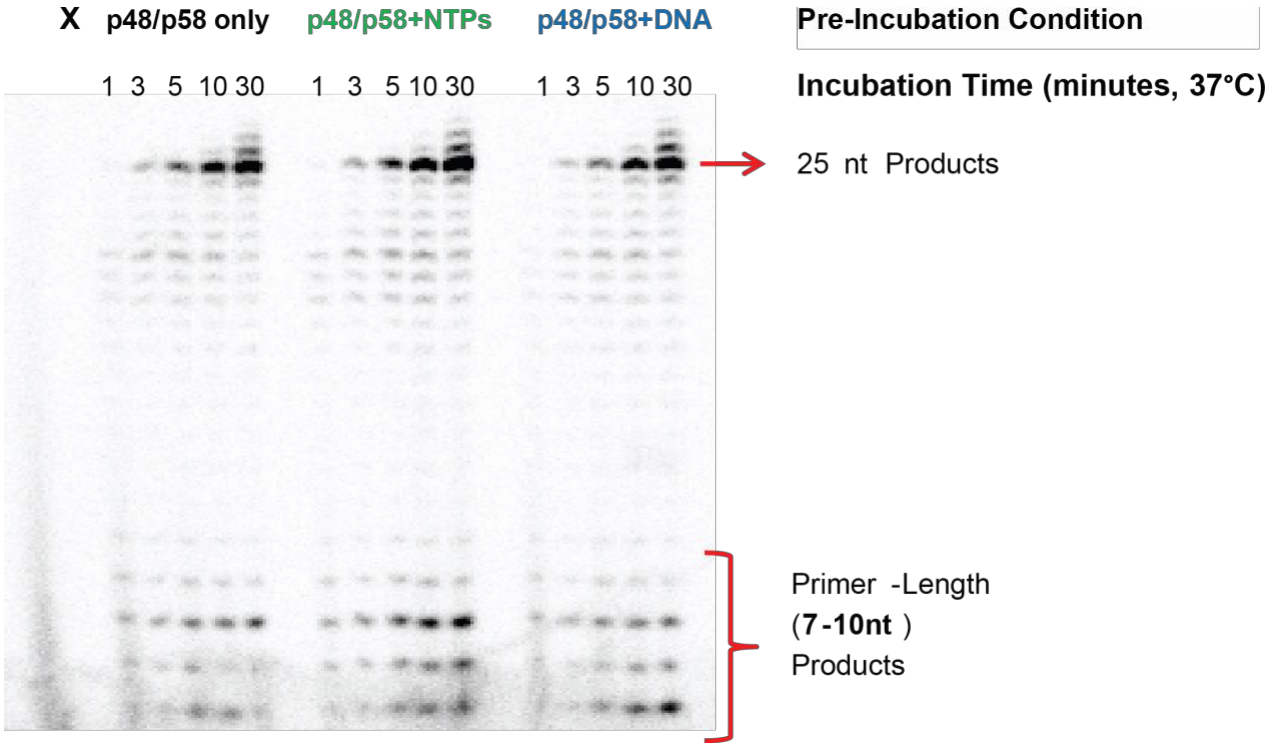

Figure S3. Substrate Binding Order Does not Affect Primase Initiation**.** Gel separation of products for three primase initiation reactions. Primase alone (p48/p58 only, grey) was added to DNA and NTPs, primase pre-incubated with NTPs was added to DNA (p48/p58 + NTPs, green), or primase pre- incubated with DNA was added to NTPs (p48/p58 + DNA, blue) to start the reaction. Pre- incubation times were 30 minutes in anaerobic conditions; all pre-incubation volumes were equal. All experiments were performed in anaerobic conditions, with 400 nM p48/p58, 250nM primed DNA, 188µM [UTP], 112µM [CTP], 1µM α-^{32}P ATP in 50mM Tris, pH 8.0, 3mM $MgCl_2$, 37°C.

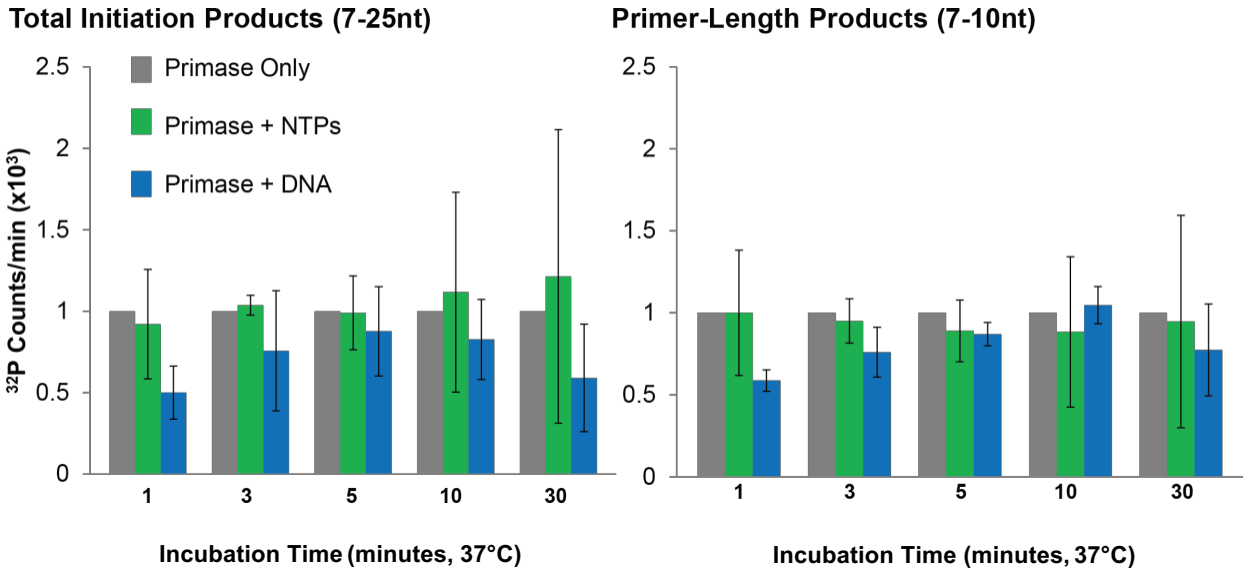

Figure S4. Quantification of primase initiation products in the pre-incubation assay. Total products (left) and primer-length (7-10nt) products (right) do not differ within error for the majority of time points assayed in the pre-incubation reaction under anaerobic conditions. This similarity suggests that substrate binding alone does not drive primase initiation, though it appears to drive elongation activity. All measurements are mean $\pm$ S.D. for n = 3 trials.

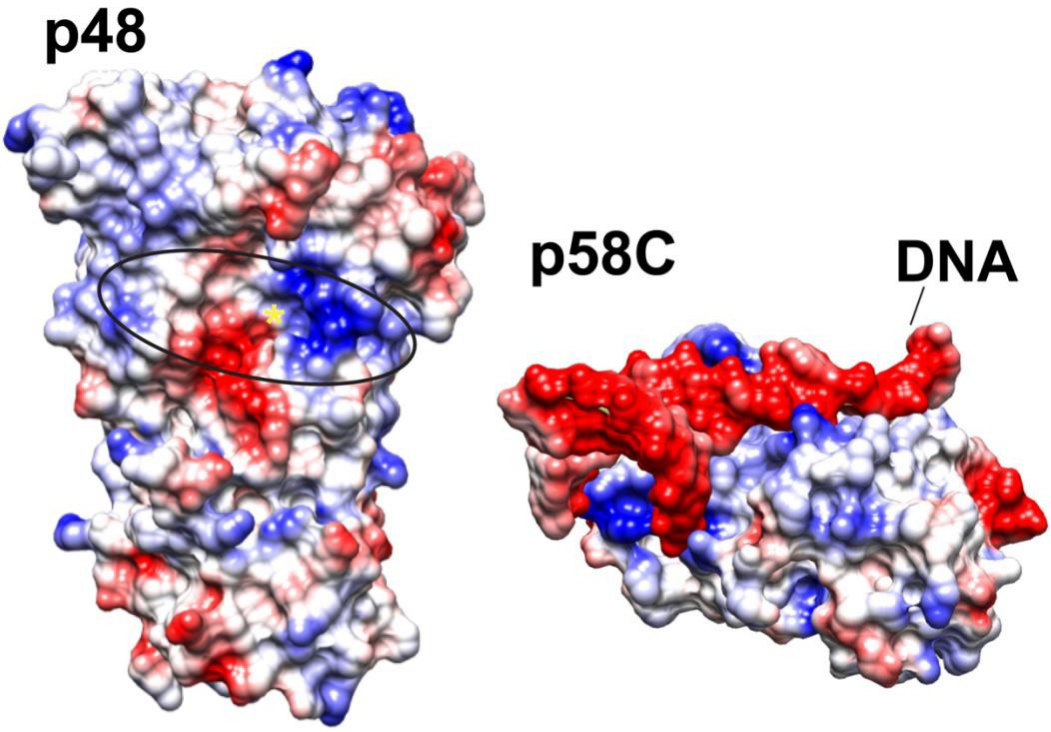

Figure S5. Electrostatic fields at the surface of p48 and p58C with a DNA substrate bound. The p48 catalytic site is indicated by a yellow asterisk. The black ring on p48 indicates where the DNA substrate and p58C need to be positioned for the initiation of priming. Coordinates used: p48 (4LIL); p58C-substate (5F0Q). Figure was generated using the MSMS package in UCSF Chimera (https://doi.org/10.1002/jcc.20084)

Primase Electrochemistry Substrate (Well-Matched)	3'-TGACTTGGGCAGGACGCAGTTGATGTACTTGTGGAG-SH-5' 5'-AGCTCTGGTACTGAACCCGTCCTGCGTCAACTACATGAACACCTC-3'
Primase Electrochemistry Substrate (Abasic Site)	3'-TGACTTGGGCAGGACGCAGTTGATGTACTTGTGGAG-SH-5' 5'-AGCTCTGGTACTGAACCCGTCCTGCGTCAACTACATGAAC_CCTC-3'
Initiation Substrate	5'-AGAAAA$(GA)_8$AAT$(A)_{25}$-3'
Elongation Substrate	3'-$(U)_{16}(T)_{15}$-5' 5'-AGAAAA$(GA)_8$AAT$(A)_{31}$-3'

Table S1. DNA substrates used for electrochemistry and DNA primase activity assays. Electrochemistry of p48/p58 and p48/p58 was performed on self-assembling monolayers of a 36-mer DNA duplex substrate with a 9-nt 5'- ssDNA overhang. A 50-nt ssDNA substrate with a single thymine base complementary to the α-^{32}P radiolabeled ATP was used in the primase initiation assay. A 2'-OMe RNA- primed ss/dsDNA substrate, containing a 31- nucleotide duplex segment and a 29- nucleotide 5'-ssDNA overhang was used to assay elongation. U = 2'-OMe rU, SH = -$(CH_2)_6$-SH.